
Claims

- 10 1. A recombinant plasmid of figure 1, wherein (1) is pET -26b(+) cloning /expression region with SEQ ID No. 1 cloned between BamH I site 198 and Nde I site 288, (2) is lac I coding sequence, (3) is pBR322 origin, (4) is Kan coding sequence, and (5) is fl origin.
- 15 2. A recombinant plasmid as claimed in claim 1, wherein the SEQ ID No. 1 is the sequence of *Bacillus subtilis* gene of figure 2, encoding conjugated bile acid hydrolase.
- 20 3. A recombinant *E.Coli* strain PTA 2456.
- 25 4. A recombinant strain as claimed in claim 3, wherein the recombinant stain produces an amount of Penicillin V acylase about 57 to 65 times more than in the ordinary conditions.
- 30 5. A recombinant strain as claimed in claim 3, wherein the strain comprises recombinant plasmid of figure 1, whereby (1) is pET -26b(+) cloning /expression region with SEQ ID No. 1 cloned between BamH I site 198 and Nde I site 288, (2) is lac I coding sequence, (3) is pBR322 origin, (4) is Kan coding sequence, and (5) is fl origin.
- 35 6. A process for the production of large amount of Penicillin V acylase using recombinant *E.Coli* strain PTA 2456, said process comprising steps of:
- 30 a. preparing a recombinant plasmid of figure 1, wherein (1) is pET -26b(+) cloning /expression region with SEQ ID No. 1 cloned between BamH I site 198 and Nde I site 288, (2) is lac I coding sequence, (3) is pBR322 origin, (4) is Kan coding sequence, and (5) is fl origin,
- 35 b. transforming the competent cells of *E. coli* with the recombinant plasmid to obtain recombinant strain PTA 2456,

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c. growing the strain in a fermentation medium for time period ranging between 4 to 18 hours at temperature ranging between 30 to 40°C, and

d. obtaining the large amount of Penicillin V acylase.

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7. A process as claimed in claim 6, wherein the amount of Penicillin V acylase obtained in the recombinant stain is about 57 to 65 times more than in the ordinary conditions.

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8. A process as claimed in claim 6, wherein the fermentation medium comprises bacto-tryptone of concentration ranging between 8-10 g/l, bacto-yeast extract of concentration ranging between 5-8 g/l, sodium chloride of concentration ranging between 3-5, and an antibiotic of concentration ranging between 30-50µg/ml.

9. A process as claimed in claim 6, wherein the *E.coli* strain is BL-21 DE3.

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